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FINE STRUCTURE AND VITAL STAINING OF OSPHRADIUM OF THE SOUTHERN OYSTER DRILL, *THAIS HAEMASTOMA CANALICULATA* (GRAY) (PROSOBRANCHIA: MURICIDAE)

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Abstract

The morphology of the osphradium of *Thais haemastoma canaliculata* (Gray) was examined using light microscopy, SEM, and TEM. The osphradium is composed of approximately 150–200 lamellae, each of which is divided into two distinct regions by a groove situated parallel to the dorsal edge of the organ. The dorsal one-fourth of each lamella is covered by dense cilia that are assumed to generate water currents about the osphradium. Ciliary tufts, located in small depressions, and numerous secretory cells are distributed uniformly on the ventral three-fourths of the lamellae. A thin tract of cilia borders the ventral edge of each lamella. The overall cellular organization is less complex than has been reported previously in other marine prosobranchs. Selective staining of putative chemoreceptors was performed using Procion Brilliant Yellow. Individual cells in the ventral region and the ventral edge of each lamella were Procion-positive. Results of this study suggest that ventral interlamellar regions and the ventral edge of each lamella are chemosensory regions, while the dorsal portion of each lamella is indifferent epithelium.

Introduction

The chemosensory function of the osphradium in prosobranch gastropods is well established (Brown and Noble, 1960; Bailey and Laverack, 1966; Bailey and Benjamin, 1968). Ultrastructural studies on several prosobranch species reveal a similar overall pattern of cellular organization, although there is little agreement on the functional interpretation of individual cell types (Welsch and Storch, 1969; Alexander, 1970; Crisp, 1973; Alexander and Weldon, 1975; Newell and Brown, 1977). Five different types of presumed sensory cells ("Sinneszelle") were identified from transmission electron microscopy of the osphradium of *Buccinium undatum* (Welsch and Storch, 1969). These five cell types, dubbed Si1-Si5 (Crisp, 1973), were thought to be either chemoreceptors or proprioreceptors. Crisp (1973) found cell types Si1-Si4 in the osphradia of five prosobranch species, but she concluded that cell types Si1 and Si2 were not sensory receptors because neither axons nor intracellular vesicles were observed. The presumed sensory receptors, Si3 (similar to a free nerve ending) and Si4 (similar to a primary receptor) were located primarily in a specialized region of the interlamellar surface (Crisp, 1973). Regional specialization of lamellar epithelium has been observed in other marine prosobranchs (Alexander and Weldon, 1975; Newell and Brown, 1977). Newell and Brown (1977), on the basis of "presynaptic vesicles," reported that some ciliated cells in the osphradium of *Bullia digitalis* might be secondary receptors. As yet ultrastructural observations have not elucidated the primary or secondary nature of receptors and no electrophysiological data have confirmed the conclusions drawn from these ultrastructural observations.

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The present investigation was initiated to study the gross morphology and ultrastructure of the osphradial epithelium of *Thais haemastoma canaliculata* (Gray) using light microscopy, SEM, and TEM and to identify putative chemoreceptor cells by vital staining with Procion Brilliant Yellow.

Materials and Methods

Adult oyster drills, *Thais haemastoma canaliculata* (Gray), were collected from the vicinity of Caminada Pass near Grand Isle, Louisiana. Drills were transported to the laboratory and placed in a 38 l aquarium at room temperature (23–25°C) and 20 ‰S (Instant Ocean® Seawater Mix).

Snails were removed from their shells by gently cracking open the shell at the base of the body whorl and severing the columellar muscle. The dorsal mantle was dissected open and folded back to expose the osphradium at the base of the ctenidium. The osphradium was dissected from the ctenidium and preserved for histological examination.

For scanning electron microscopy (SEM), osphradia were fixed overnight with 2.5% gluteraldehyde in 0.2 *M* sodium cacodylate-sucrose buffer (585 mOsm; pH = 8.0). Specimens were rinsed in distilled water to remove all buffer salts, dehydrated in acidified 2,2-dimethoxypropane, critical-point dried in CO_2 and coated with 200Å of Au/Pd. Osphradia were examined at 25kV with a Hitachi S-500 SEM.

For transmission electron microscopy (TEM), osphradia were fixed overnight with 2.5% gluteraldehyde in 0.2 *M* sodium cacodylate-sucrose buffer (585 mOsm; pH = 8.0) and post-fixed for 2 h in 1% OsO_4 buffered in 0.2 *M* sodium cacodylate (pH = 8.0). Specimens were dehydrated in ethanol and embedded in Epon. Ultrathin sections were placed on copper grids and contrasted with uranyl acetate-lead citrate. Sections were examined with a JEOL 100-CX TEM at 80 kV.

Vital staining of epithelial receptor cells in the osphradium was accomplished by a modification of the method of Holl (1981). Live drills acclimated to 7.5 ‰S were anesthetized by chilling on ice. The shell of each snail was gently cracked at the body whorl, immediately over the osphradium. The mantle edge was cut and reflected and the exposed osphradium was periodically irrigated for 30 minutes with a 4% solution of Procion Brilliant Yellow in 0.1 *M* KCl. For four species of freshwater fish, Holl (1981) used a 0.01 *M* KCl staining solution, a concentration we considered too low for an estuarine gastropod. *Thais haemastoma canaliculata* can be readily acclimated to low salinity (Garton and Stickle, 1980), therefore a 0.1 *M* KCl solution (200 mOsm/kg) was used so that osmotic differences between the staining solution and the hemolymph would be minimized in low salinity acclimated snails (7.5 ‰S equals 223 mOsm/kg). Excess stain was flushed away with 7.5 ‰S sea water. The osphradium was post incubated at room temperature (24°C) for 30 minutes, removed from the snail and fixed in buffered formalin (pH = 6.5). The specimen was dehydrated in alcohol, cleared in xylene and embedded in paraffin. Sections, 7 μm, were mounted on slides and the paraffin removed with xylene. Sections were coated with immersion oil and viewed at 390–490 nm (mirror—510 nm; suppression—515 nm) with an epifluorescence light microscope equipped with a Leitz H2 filter block. Non-stained osphradia were examined as controls.

Results

SEM and TEM observations

The general morphology of the osphradium in *Thais haemastoma canaliculata* is typical of prosobranch gastropods, being bipectinate and containing approximately

150–200 lamellae (Fig. 1). The pseudostratified epithelium is separated from the central region of the lamella by a distinct basal lamina. Connective tissue, muscle fibers, nerves, and blood spaces are present in the central region of each lamella (Fig. 2). In the live snail the lamellae are active and capable of independent movement.

In *Thais haemastoma canaliculata* a groove 10–15 μm wide and 5 μm deep runs parallel to the dorsal edge of each lamellae and separates the lamellar epithelium into two distinct regions (Figs. 3, 4). A thin tract of cilia is present along the ventro-lateral edge of each lamella (Figs. 3, 5). The epithelium dorsal to the groove is composed entirely of a single layer of ciliated cells. There are relatively few mitochondria in these cells, and the mitochondrial cristae are diffuse, indicating a relatively low metabolic rate (Munn, 1974). Ciliary rootlets are short and many microvilli cover the cell surface. The epithelium ventral to the lamellar groove, occupying approximately

FIGURE 1. SEM of the anterior portion of the osphradium (Os) showing its relation to the ctenidium (Ct) and incurrent siphon (S). Me–mantle epithelium; R–raphe.

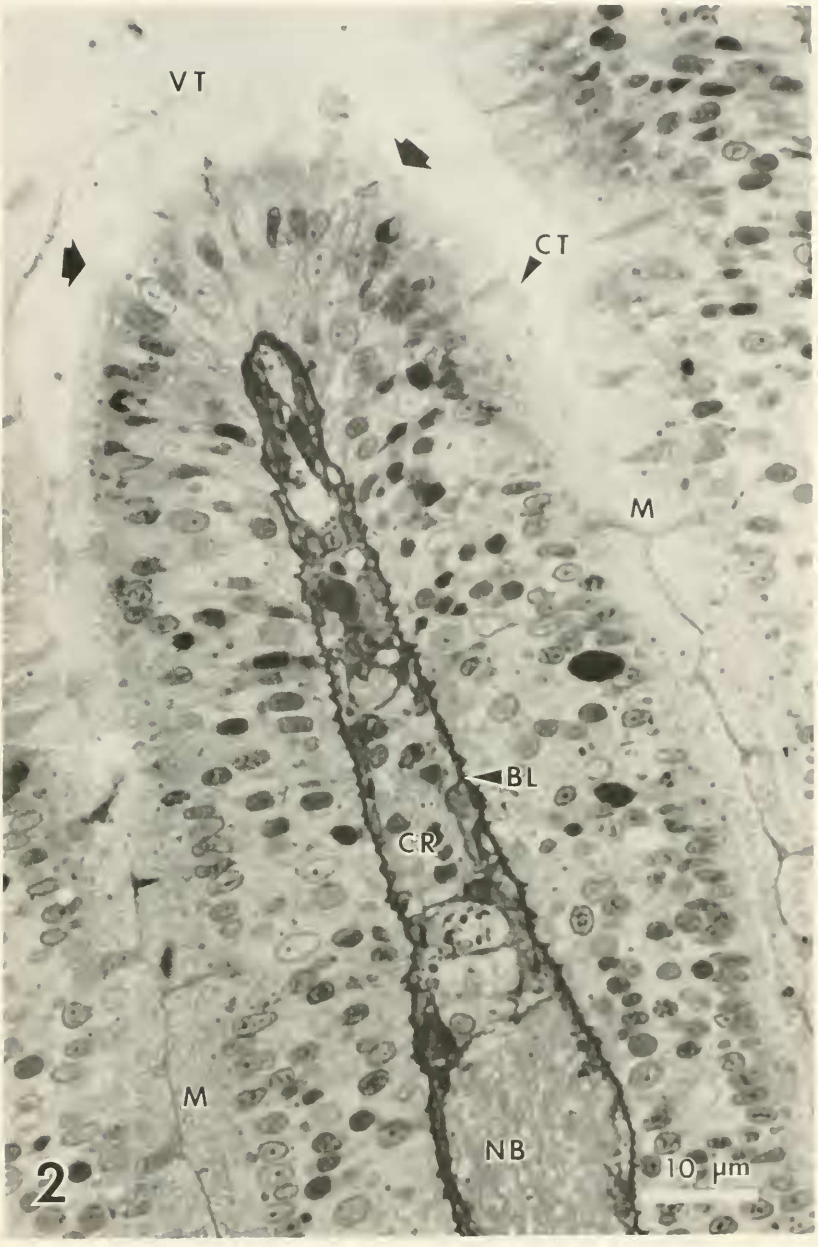


FIGURE 2. Longitudinal thin section (1 μm) of a single osphradial lamella stained with Paragon. BL–basal lamina; CT–ciliary tuft; CR–central region; M–mucus layer; NB–nerve bundle; VT–ventral ciliary tract (between arrows).

three-fourths of the surface area of each lamella, contains uniformly distributed, tufted ciliated cells (Figs. 6, 7). These cilia originate from cells lying depressed between neighboring cells. Numerous mitochondria with well developed cristae are concentrated

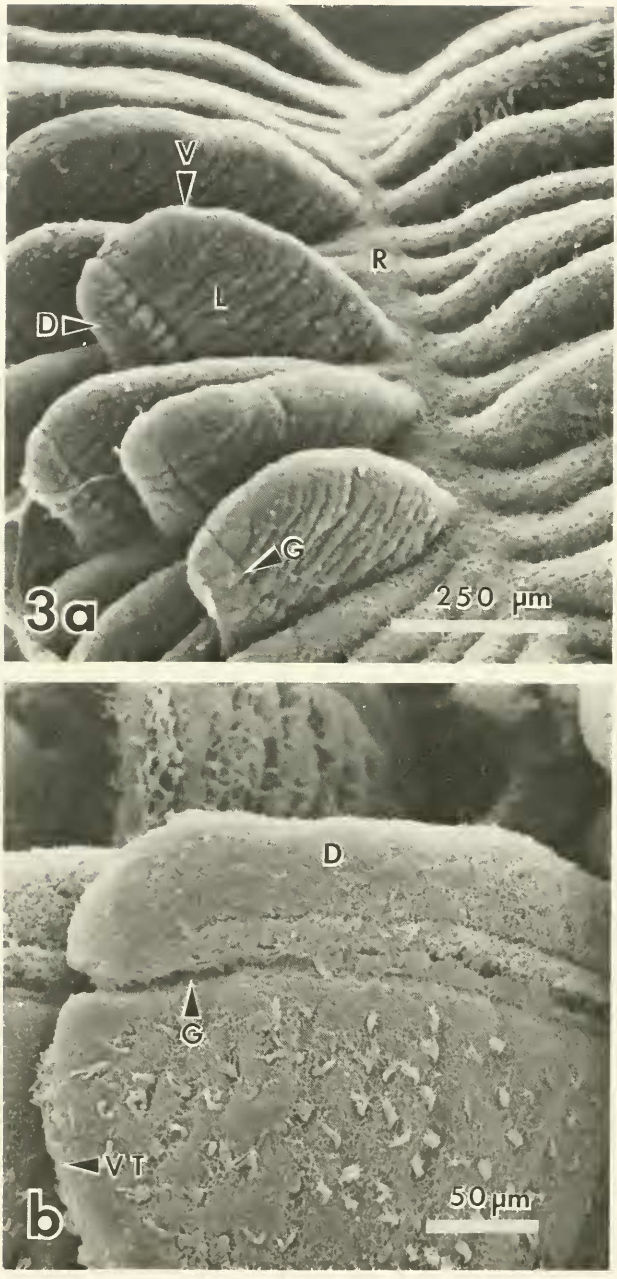


FIGURE 3a. SEM of an inverted osphradium showing directional references for the lamella. Note the unequal extension of individual lamellae. D–dorsal edge of lamella; G–lamellar groove; L–lamella; R–raphe; V–ventral edge of lamella. b. Lateral view of single lamella (SEM), orientation reversed from a. D–dorsal edge; G–lamellar groove; VT–ventral tract of cilia.

at the apical end of the ciliated cell (Fig. 8). Long ciliary rootlets extend basally through the aggregate of mitochondria. Ciliary tufts project laterally into the interlamellar space. The interlamellar space is filled with mucus and membrane-bound

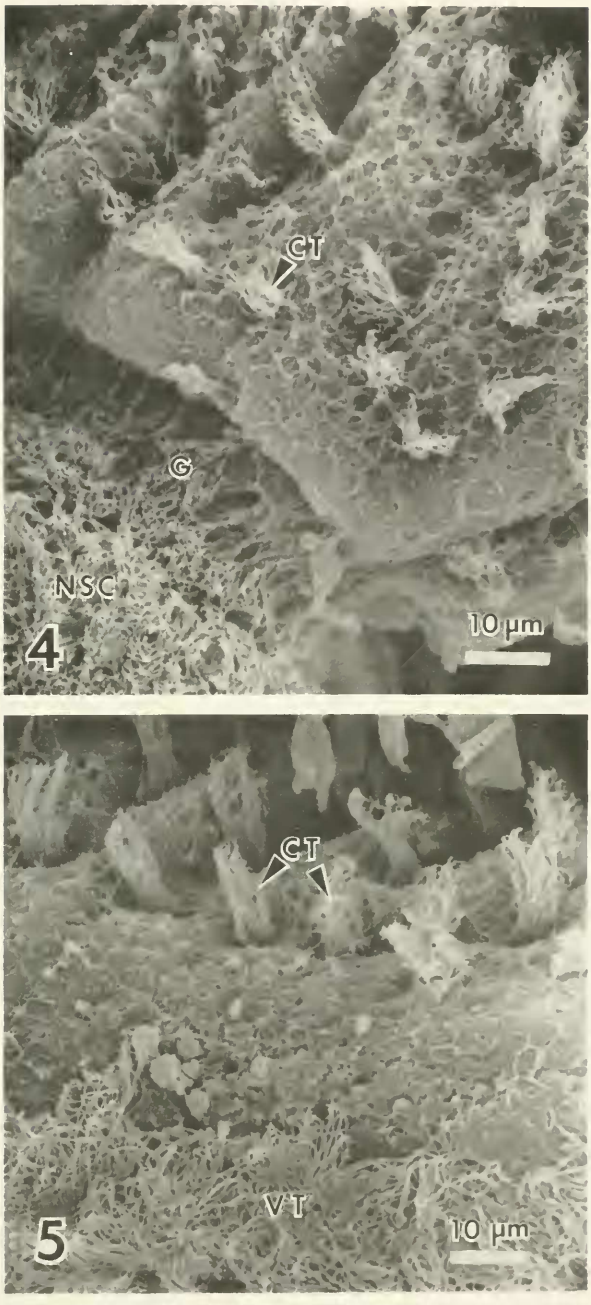


FIGURE 4. Transitional region on the dorsal edge of a single lamella (SEM). CT–cilia tuft; G–lamellar groove; NSC–non-sensory cilia.

FIGURE 5. Transitional region (SEM) on the ventral edge of a single lamella. CT–cilia tuft; VT–ventral tract of cilia.

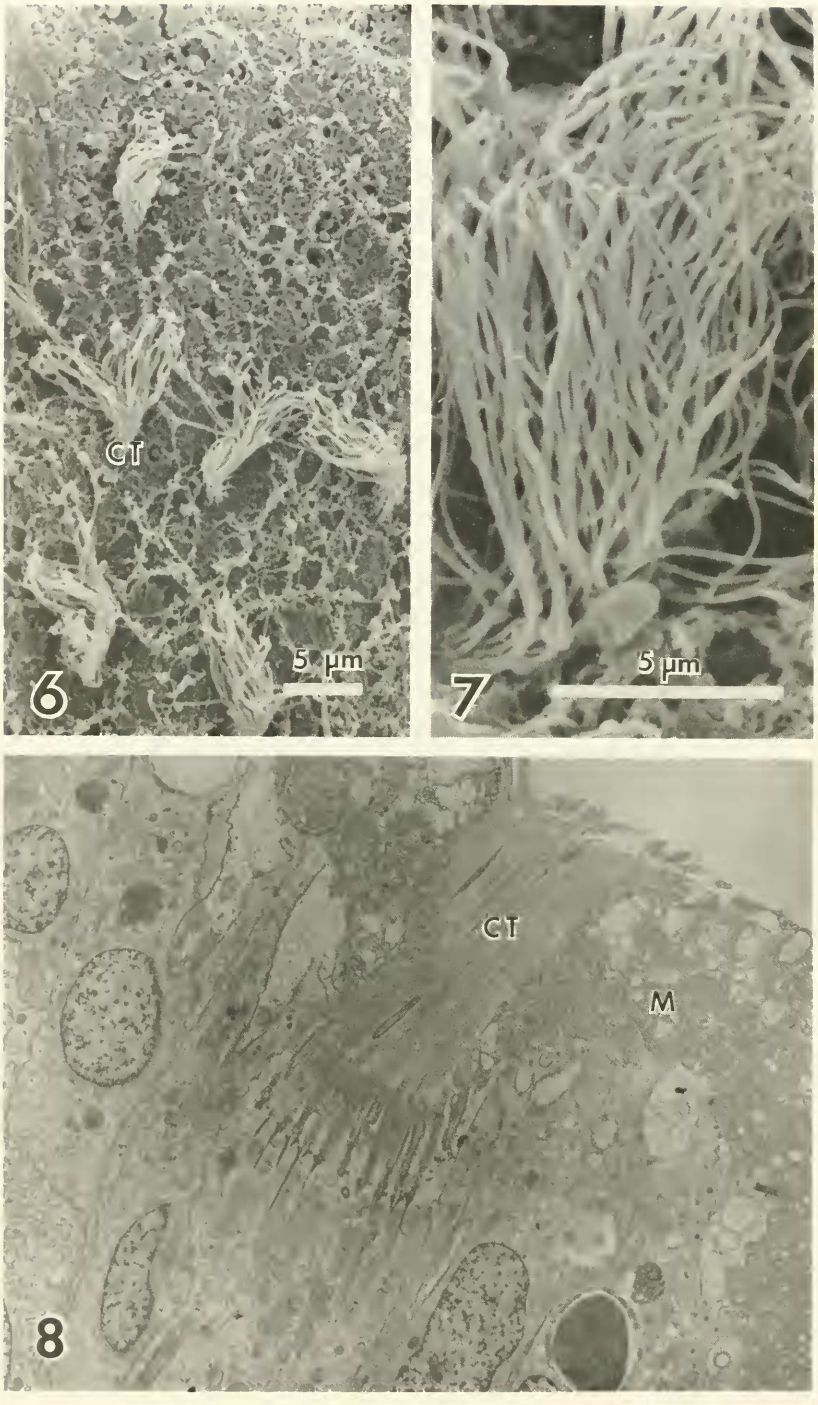


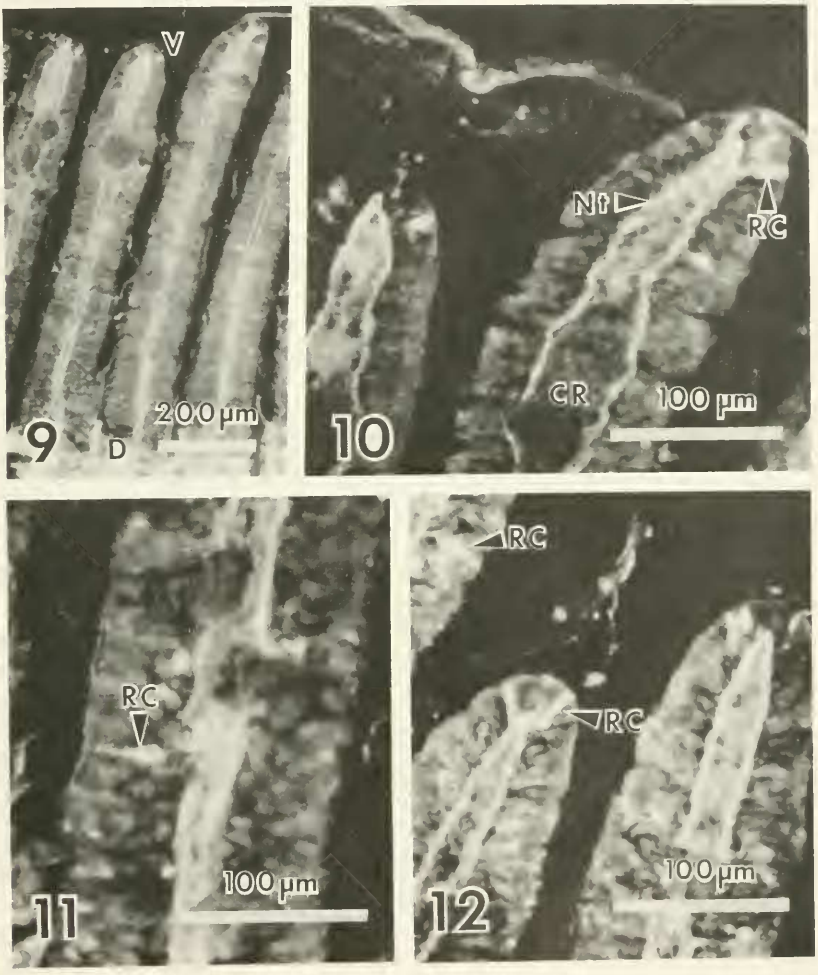
FIGURE 6. SEM of ciliary tufts (CT) on the interlamellar surface.
FIGURE 7. SEM of individual ciliary tuft in Figure 6.
FIGURE 8. TEM of ciliary tuft (CT) projecting into mucus layer (M). Magnification = 4125×.

secretory vesicles produced by the surrounding non-ciliated cells present in the ventral region of the lamellae (Fig. 8).

All cilia observed by TEM, regardless of location, were of the $9 \times 2 + 2$ fibril arrangement. Cilia were traced distally in many sections and no deviation from the $9 \times 2 + 2$ arrangement was observed. Nerve tracts were located in the central region of the lamellae but no axons or synapses bridging the basal lamina were observed.

Vital staining

Selective staining of individual receptor cells is shown in Figures 9–12. Epithelial cells present in the region ventral to the groove and along the ventro-lateral tract of



FIGURES 9–12. Fluorescent light micrographs of Procion Brilliant Yellow stained material. FIGURE 9. Low magnification of osphradial leaflets. D dorsal; V ventral. FIGURE 10. Procion-positive cells (RC) at ventral tip of lamella. CR–central region; Nt–nerve tract. FIGURE 11. Procion-positive cell (RC) in interlamellar region. FIGURE 12. Example of a Procion-positive cell (RC) at ventral or distal region of lamella.

cilia stained as well as nerve tracts in the central region (Figs. 9–12). Although the individual cells that were stained by Procion Brilliant Yellow could not be identified as specific cell types observed in the SEM and TEM material, their location suggests that tufted ciliated cells along the interlamellar surface and ciliated cells of the ventro-lateral tract were stained.

Discussion

The osphradial lamella in *Thais haemastoma canaliculata* (Gray) is divided into two morphologically distinct regions. This division has been observed in other prosobranchs (Crisp, 1973; Newell and Brown, 1977; Altner and Prillinger, 1980). Welsch and Storch (1969) and Crisp (1973) reported that the presumed sensory receptors are concentrated in the epithelium adjacent to the "transition zone" or groove on the lamella. In *Conus flavidus* the arrangement is more complex. Alexander (1970) and Alexander and Weldon (1975) observed presumptive receptor cells along interdigiform grooves on the interlamellar surfaces of the osphradial lamellae in *Conus flavidus.* These cells were also located in small depressions, similar to those in *T. haemastoma canaliculata.* Hackney *et al.* (1983) identified tufted, ciliated cells as putative chemoreceptors on the pallial tentacles of the limpet *Patella vulgata.* The gross morphology of these tufted cells is similar to that of the tufted ciliated cells observed in this study. Our observations imply that rather than being restricted to a specialized "transition zone," chemosensory cells are distributed over a major part of the osphradial lamella in *T. haemastoma canaliculata.*

Results of the vital staining in *Thais haemastoma canaliculata* also indicate that sensory cells (possibly chemosensory) are uniformly distributed across the interlamellar surface and ventro-lateral edge of the lamella. Procion-positive cells are denser along the ventral edge than across the interlamellar surface. Ventral edges of lamellae project into the inhalant water stream (the osphradium is located on the dorsal wall of the mantle cavity adjacent to the ctenidium), providing maximal exposure to environmental stimuli. After contacting ventral edges, water currents pass through mucus-filled interlamellar spaces, then along the dorsal edge of lamellae, adjacent to the mantle epithelium. Any chemosensory cells present in the dorsal region would come into contact with odorant molecules last.

Deviations from the $9 \times 2 + 2$ fibril arrangement in osphradial cilia have been observed in prosobranchs (Crisp, 1973). Crisp (1973) reported $(8 + 1)$, $(8 + 0)$, $(7 + 1)$, $(7 + 0)$, and $(6 + 1)$ arrangements. Other ciliary modifications for a putative chemoreceptor cell have been observed in an opistobranch (Davis and Matera, 1982; Matera and Davis, 1982). "Discocilia" were observed only in chemosensitive regions of the body of *Pleurobranchaea californica* (Davis and Matera, 1982). SEM and TEM observations in our study revealed no specialized modifications of cilia in different regions of the osphradial lamellae that might suggest chemosensory function. All cilia observed were of the $9 \times 2 + 2$ fibril arrangement.

Both primary and secondary receptors have been reported in osphradial receptors (Crisp, 1973; Newell and Brown, 1977). Crisp (1973) speculated that the Si3 and Si4 cell types are primary receptors, although no axons were observed. Given the volume of epithelial cells compared to axon sizes (tenths of microns), the probability that we could observe axonal connections in TEM material is quite low. Crisp (1973) reported no evidence for secondary receptors (synaptic vesicles); however, Newell and Brown (1977) reported chemical synapses in the osphradial epithelium of *Bullia* associated with tufted ciliated cells, although no high magnification figure of the vesicles was provided. In invertebrates, the great majority of sensory cells are primary receptors;

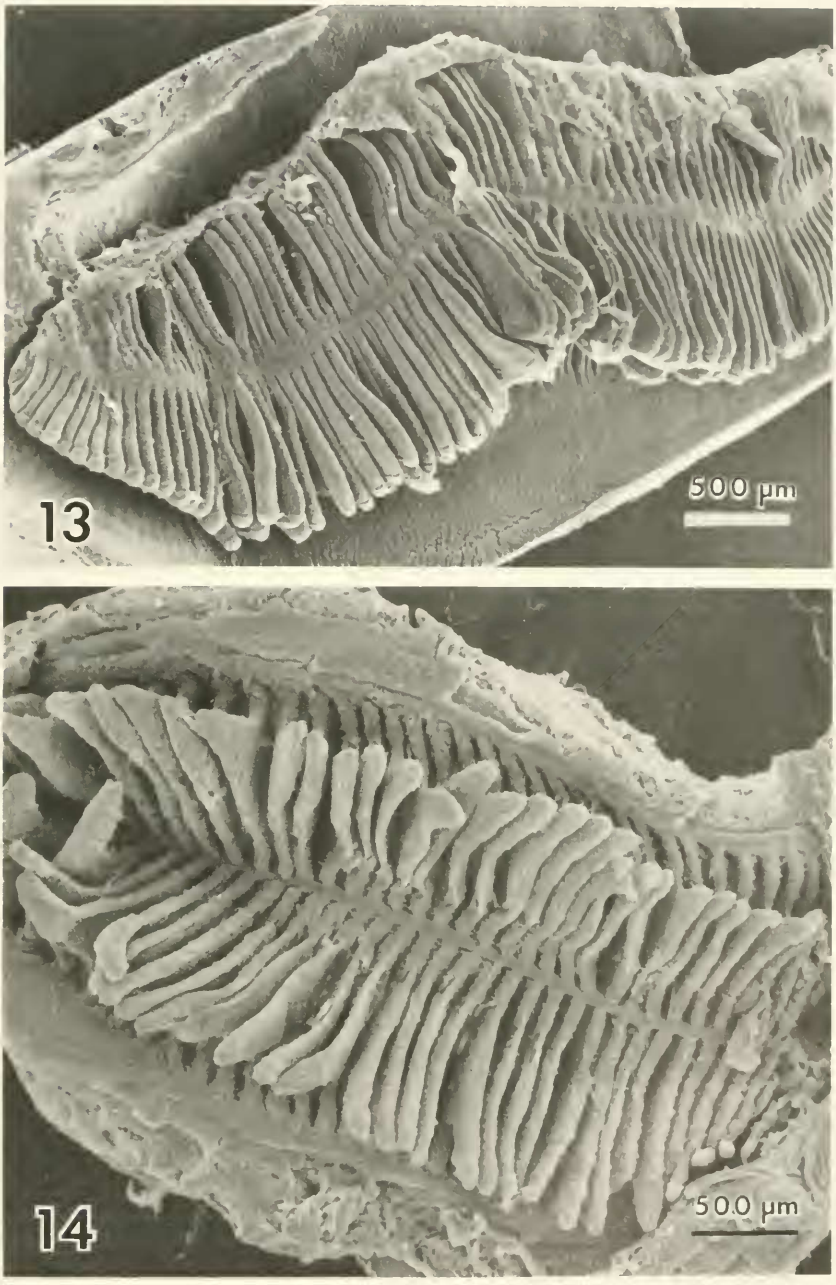


FIGURE 13. Scanning electron micrograph (SEM) of the ventral surface of the osphradium of the southern oyster drill, *Thais haemastoma canaliculata* (Gray). The osphradium is suspended from the roof of the mantle cavity and the lamellae project ventrally into the incurrent water stream. Anterior is to the left.

FIGURE 14. SEM of the dorsal surface of the olfactory rosette of the channel catfish, *Ictalurus punctatus*. The rosette is attached to the floor of the olfactory capsule and the lamellae project dorsally into the olfactory chamber. Anterior is to the right.

development of secondary receptors is considered a vertebrate characteristic (Bullock and Horridge, 1965). Nerve tracts associated with putative (chemo?)-receptors were observed in the Procion Brilliant Yellow stained material, although none were observed in TEM or fractured SEM specimens. Transport of the stain along the nerve tracts suggests that the selectively stained epithelial cells are primary receptor cells. Also, no synapses or intracellular vesicles indicative of secondary receptors were observed.

Cellular organization of the osphradial epithelium in *Thais haemastoma canaliculata* is less complex than has been reported for other prosobranchs (Crisp, 1973; Newell and Brown, 1977; Altner and Prillinger, 1980). The results of this study suggest a functional separation of different epithelial regions of each lamella (sensory *versus* non-sensory) similar to that found in many teleosts (Yamamoto, 1982). The gross morphology of the prosobranch osphradium (Fig. 13) and the teleost olfactory organ (Fig. 14) are strikingly similar, suggesting convergent evolution. Both organs are composed of numerous, laterally radiating lamellae organized into specialized sensory and indifferent epithelial regions (Caprio and Raderman-Little, 1978; Yamamato, 1982).

Because definitive axonal connections or synapses between ciliated cells and nerve fibers have not yet been identified, the assignment of sensory function to cells in the prosobranch osphradium is currently based solely on cytological observations and selective vital staining. Although the results of our and other studies have identified putative chemoreceptors, electrophysiological investigations will be necessary to confirm and elucidate structure-function relationships of receptor cells and specialized lamellar regions of the prosobranch osphradium.

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